

2019-01-24

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von Engelhardt, NB

<http://hdl.handle.net/10026.1/13257>

10.1242/jeb.187005

Journal of Experimental Biology

Company of Biologists

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Effects of the maternal and current social environment on female body mass and reproductive traits in Japanese quail (*Coturnix japonica*)

Esther MA Langen^{*a,b,1}, Vivian C Goerlich-Jansson^{a,b,1}, Nikolaus von Engelhardt^{a,2},

^{*}Corresponding author: Department of Animals in Science and Society, Utrecht University, Yalelaan 2, 3508 TD Utrecht, The Netherlands. E-mail address: emalangen@gmail.com.

^a Department of Animal Behaviour, Bielefeld University, Morgenbreede 45, 33615 Bielefeld, Germany

^b Department of Animals in Science and Society, Utrecht University, Yalelaan 2, 3508 TD Utrecht, The Netherlands

¹ Present address: Department of Animals in Science and Society, Utrecht University, Yalelaan 2, 3508 TD Utrecht, The Netherlands. E-mail address: v.c.goerlich-jansson@uu.nl

² Present address: Faculty of Science and Engineering, University of Plymouth, Drake Circus, Plymouth Devon PL4, 8AA Plymouth, United Kingdom. E-mail address: nvengelhardt@gmx.de

Keywords

Transgenerational effects; group size; reproductive investment; steroid hormones; physiology; morphology

Summary statement

The maternal and current social environment have interacting effects on body mass of female Japanese quail, but only the current social environment affects reproductive traits and offspring mass at hatching.

Abstract

The social environment of breeding females can affect their phenotype with potential adaptive maternal effects on offspring experiencing a similar environment. We housed Japanese quail (*Coturnix japonica*) females in two group sizes (pair of two vs. groups of four) and studied the effects on their offspring under matched and mismatched conditions. We measured F1 body mass, reproduction, and plasma levels of androgens and corticosterone. F1 group housing led to an increase in body mass. In addition, F1 group housing had a positive effect on weight in daughters of pair-housed P0 females only, which were heaviest under mismatched conditions. At the time of egg collection for the F2 generation, F1 group females were heavier, irrespective of the P0 treatment. F1 females in groups laid heavier eggs, with higher hatching success, and produced heavier offspring, most likely a maternal effect of F1 mass. F1 plasma hormones were affected neither by the P0 nor the F1 social environment. These results contrasted with effects in the P0 (reported previously), in which plasma hormone levels differed between social environments, but not mass. This may be due to changes in adult sex ratios since P0 females were housed with males, whereas F1 females encountered males only during mating. Our study demonstrates potentially relevant mismatch effects of the social environment on F1 weights and maternal effects on F2 offspring, but further study is needed to understand their adaptive significance and physiological mechanisms.

Introduction

Effects of the maternal social environment on female physiology, reproduction and offspring phenotype have been described in various species, including birds and mammals (Groothuis et al., 2005; Guibert et al., 2010; Kaiser and Sachser, 2005, 2009). Maternal effects can act as mechanisms of adaptive transgenerational plasticity to optimally prepare offspring phenotype for their future environment. This can be tested by studying the consequences for offspring experiencing an environment that matches or mismatches the maternal environment (Burgess and Marshall, 2014; Marshall and Uller, 2007; Uller et al., 2013). This study investigates the transgenerational effects of maternal social group size on offspring housed under either matched or mismatched social conditions in an avian species, the Japanese quail (*Coturnix japonica*).

Behaviour, physiology and reproduction can be affected by the social environment, such as density, group size, social rank, mate attractiveness or adult/operational sex ratio (Alonso-Alvarez et al., 2012; Asghar Saki et al., 2012; Benyi et al., 2006; Both, 1998; Both et al., 2000; Clutton-Brock and Huchard, 2013; Cunningham and Russell, 2000; Dewsbury, 1982; Ellis, 1995; Fowler, 1981; Rodenhouse et al., 2003; Schubert et al., 2007; Sillett et al., 2004; Stockley and Bro-Jørgensen, 2011; Székely et al., 2014; Uller et al., 2005). Effects of the social environment on female endocrine physiology and body mass (Bonenfant et al., 2009; DeVries et al., 2003; Eisenegger et al., 2011) provide proximate mechanisms through which reproduction and offspring can be affected. In birds, increasing group size, for example, is thought to exacerbate intraspecific competition which can affect body mass (Asghar Saki et al., 2012; Keeling et al., 2003; Onbaşlılar and Aksoy, 2005) and circulating levels of steroid hormones such as corticosterone and androgens (Cantarero et al., 2015; Cunningham et al., 1987; Koelkebeck and Cain, 1984; Langmore et al., 2002; Mazuc et al., 2003; Onbaşlılar and Aksoy, 2005; Raouf et al., 2006; Smith et al., 2005). In Japanese quail, frequent changes in the group composition of breeding females are thought to reflect increased social densities and lead to elevated plasma corticosterone concentrations (Guibert et al., 2010). In contrast,

Japanese quail females housed in pairs had higher circulating androgen levels and tended to have higher circulating corticosterone levels than group-housed females (Langen et al., 2017). Such effects of the social environment on female physiology and body mass and condition may affect their ability to invest in reproduction, resulting in changes in the quality or quantity of eggs produced or the quality or quantity of the offspring (Christians, 2002; Drent and Daan, 1980; Lim et al., 2014; Ronget et al., 2018; Sockman et al., 2006). Studies have reported both positive and negative correlations between measures of reproduction and circulating androgens (positive: Cain and Ketterson, 2012; Langmore et al., 2002; Sandell, 2007; negative: de Jong et al., 2016; López-Rull and Gil, 2009; Rutkowska et al., 2005; Rutkowska and Cichoń, 2006; Veiga and Polo, 2008) and glucocorticoids (positive: Bonier et al., 2009b; Burtka et al., 2016; Ouyang et al., 2011, 2013; negative: Angelier et al., 2010; Bonier et al., 2009b; Ouyang et al., 2011, 2013; Silverin, 1986; Vitousek et al., 2014).

Effects of the social environment on female physiology and reproductive investment can lead to effects on offspring development and fitness. Kaiser et al. (2003) found in guinea pigs (*Cavia aperea*), for instance, that maternal social instability resulted in decreased maternal plasma androgen concentrations and affected offspring behaviour and physiology. Daughters of unstable mothers were masculinized in their behaviour and had increased plasma androgen concentrations during adulthood, whereas sons were infantilized. In American red squirrels (*Tamiasciurus hudsonicus*), higher maternal social densities increased maternal corticosterone and offspring growth rates (Dantzer et al., 2013). In Japanese quail (*Coturnix japonica*), maternal social instability reduced offspring growth during the first weeks of life (Guibert et al., 2010). Maternal effects on growth and physiology may influence offsprings' future reproduction since an individual's reproductive performance often depends on its body condition and/or endocrine status (Burtka et al., 2016; Correa et al., 2011; de Jong et al., 2016; Devries et al., 2008; Festa-Bianchet et al., 1998; López-Rull and Gil, 2009; Milenkaya et al., 2015; Ouyang et al., 2011, 2013; Rutkowska et al., 2005; Veiga and Polo,

2008). However, the adaptive significance of maternal effects induced by social stimuli is still insufficiently understood.

In the present study we investigate the potential interactive effects of the maternal and offspring social environment. Females of the parental (P0) generation were housed in pairs (one female and one male) or in groups (three females and one male) and allowed to reproduce (Langen et al. 2017). The females of the offspring (F1) generation were similarly housed in either pairs of two females or groups of four females, with daughters from the two maternal conditions evenly allocated to the two F1 social conditions. This allowed us to investigate the effects of the P0 social environment, the F1 female's own social environment, and their interaction on physiology (body mass and circulating levels of corticosterone and androgens) and reproduction (egg production, egg mass, fertilization rates, hatching success, and offspring mass). We assessed the sensitivity of the F1 female's hypothalamic-pituitary-adrenal (HPA) axis using a standardized restraint stress challenge (Wingfield et al., 1995) and assessed the responsiveness of the hypothalamic-pituitary-gonadal (HPG) axis using a gonadotrophin-releasing hormone (GnRH) challenge (Jawor et al., 2006; Peluc et al., 2012). This enabled us to investigate whether effects on reproductive performance reflect physiological changes during reproduction (e.g., Angelier et al., 2010; Bonier et al. 2009b, Burtka et al., 2016, Cunningham et al., 1987, Ouyang et al., 2011, 2013).

Adaptive effects of the maternal social environment should prepare their offspring for the social environment anticipated by the mother's social experience. We therefore expected F1 female offspring to become heavier and reproduce better under social conditions matching the maternal environment compared to the female offspring housed under mismatched social conditions. Social density or group size are frequently positively correlated with circulating androgen or corticosterone levels (Cunningham et al., 1987; Mazuc et al., 2003; Onbaşlılar and Aksoy, 2005; Raouf et al., 2006; Smith et al., 2005) This would suggest higher plasma androgen or corticosterone concentrations in group-housed females compared to pair-housed females. However, since we previously found that female Japanese quail housed in pairs had

higher circulating androgen levels and tended to have higher circulating corticosterone levels compared to females housed in groups, we expected that the reverse might also be found.

Materials and methods

Ethics statement

All experimental procedures were approved by the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), Recklinghausen, Germany (licence number 84-02.04.2013-A127). Animal facilities were approved for keeping and breeding Japanese quail for research purposes by the local government authority responsible for health, veterinary and food monitoring (Gesundheits-, Veterinär- und Lebensmittelüberwachungsamt Bielefeld, Germany).

Origin of the parental generation

The eggs from which the parental generation hatched were provided by the INRA in Nouzilly, France (Experimental unit 1295 (UE PEAT) and UMR 85, Physiologie de la Reproduction et des Comportements, INRA-CNRS-IFCE-Université de Tours, Val de Loire Center, Nouzilly, France). The eggs were laid by females from a non-selected control line, bred next to quail lines selected for low or high social reinstatement (Mills and Faure, 1991).

Social environments

Females were housed under two different social conditions shortly before sexual maturity: P0 females were housed in pairs (one female with one male) or in groups (three females with one male) and F1 females were housed in pairs (two females, one offspring from each of the P0 treatments) or in groups (four females, two offspring from each of the P0 treatment). The birds were placed in the experimental social conditions at the age of 29 days in the P0 generation

(Langen et al., 2017) and 24 days in the F1 generation (Fig. 1), about two weeks before the onset of egg laying. At that time the birds were still unfamiliar with each other. Siblings and half-siblings (in the P0) or cousins (in the F1) were never housed in the same cage. F1 males (n=15, all offspring from the P0 pair treatment) were housed in single cages and only encountered females for mating. Males were not housed with females in the F1 generation to avoid injury to the females which could result from high copulation frequency when housed in pairs (see Langen et al., 2017).

In the P0 generation, 17 pair-housed females and 20 group-housed females produced F1 offspring (Langen et al., 2017). Thirteen of the pair-housed females and 13 of the group-housed females produced the 53 daughters used in the current experiment. These F1 females were allocated to 16 pairs and 7 groups, mixing offspring from both maternal treatments where possible, so that they were exposed to the same current social treatment (see also Table S1). We thus created four different treatments in the F1 generation, representing all combinations of the P0 and F1 social conditions: daughters from pair-housed mothers housed in pairs ($P_{P0}P_{F1}$, n=16), daughters from pair-housed mothers housed in groups ($P_{P0}G_{F1}$, n=11), daughters from group-housed mothers housed in groups ($G_{P0}G_{F1}$, n=13), and daughters from group-housed mothers housed in pairs ($G_{P0}P_{F1}$, n=13). Three pair cages and three group cages contained females that were not used for the experimental tests, but served as cage mates for the experimental birds (see also Table S1). These seven females were the offspring of P0 birds that had been excluded from the experiments due to aggression (for more information, see Langen et al., 2017). For details on sample sizes, see Table 1.

Due to aggression, we had to separate 11 pairs and 4 groups in the F1 generation over the course of the experiment. Of the 11, 10 pairs were separated using a wire mesh so that visual, acoustic and limited tactile interaction was still possible, and they were kept in our experiment. One pair was completely separated and removed from the experiment because one of the females had wounds that were unlikely to heal within a few days, constituting a pre-established humane endpoint. The four groups had to be fully separated because it was not

possible to use a wire mesh in their cage to keep them apart and allow visual, acoustic and tactile interaction. We included only data from before the separation of the one pair and the four groups, and after separation all females from the respective cage were excluded. In addition, for some females measurements were not included in certain analyses due to missing samples (for one female cortisol measurements from the stress protocol were missing and for two females androgen measurements from the GnRH challenge were missing because blood sampling failed; for one female no reproductive measures could be calculated because she did not lay any eggs). Therefore each measurement had a different sample size (for exact sample sizes, see Tables 1-2 and Fig. 2, 4 and 5). For more details on when the birds were separated, see Table S1.

Animal husbandry

All birds were housed in two adjacent rooms in the P0 generation (Langen et al., 2017) and three adjacent rooms in the F1 generation (two rooms for the females and one room for the males). All rooms had artificial lighting and ambient temperature, with a minimum temperature of 20°C. Main lights were set to a 14:10h light-dark cycle (lights on at 5 am), except for the first day and night after hatching when lights remained on for 24 hours. Cages never faced each other to prevent visual contact between birds from different cages, but acoustic and olfactory communication was possible.

In the P0 generation, pairs were kept in cages measuring 75 x 80 x 40 cm, groups in cages measuring 150 x 80 x 40 cm. The adult F1 females were all kept in cages measuring 150 x 80 x 40 cm, irrespective of their social conditions. Males were housed in cages measuring 75 x 80 x 40 cm. Birds were kept on wood shavings, and all cages contained a sand bath and one shelter hut per bird. Food (GoldDott Hennenmehl, Derby Spezialfutter GmbH, Münster, Germany) and water was provided ad libitum. On a weekly basis, the standard diet was supplemented with mealworms and shell grit.

Females were weighed before they were housed in their adult social condition on day 24, and on days 30, 37, 44, 61, 90 and 97.

Mating

Females of the F1 generation were housed in single-sex groups but had temporary access to males for mating (see Fig. 1). In each mating session, males and females were together for 20 minutes. Fifteen males, all sons of pair-housed females, were used in total, and females were always paired with the same unrelated male (not sharing the same grandparents). Each male was paired with four different females, one from each combination of the P0 and F1 social conditions, except for one male who was only paired to $P_{P0}P_{F1}$ females. On days 55-56 males were introduced into the home cages of the females and allowed to mate for 20 minutes. Since the male was unable to copulate with the two or four females in a cage within such a short time period, we subsequently paired him with one female at a time in the next mating sessions. Each female was paired twice a week, and each male was paired with the same two females per day but in alternating order. Furthermore, we started the mating sessions with a different male and female every day so that the pairing order was randomised for males as well as females. On days 57-58 and 61-64, females and males were paired in a neutral mating cage between 08:00 and 17:00. Thereafter, on days 68-69, 70-71, 76-77, 78-79 and 82-83, females were introduced to their male's home cage from 10:00-12:30.

Egg collection for the F2 generation, incubation and hatching

Eggs for the F2 generation were collected on days 80 - 87. All eggs were stored at 16°C until the end of the collection period (storage time ranging between 1 - 7 days) when incubation started. All eggs were incubated at the same time in a HEKA-Euro-Lux II incubator (HEKA-Brutgeräte, Rietberg, Germany). Incubation was done in complete darkness to avoid the

effects of light on development (Archer and Mench, 2014). From incubation day 1 to day 14, the temperature was set at 37.8°C, humidity at 55%, and the eggs were turned every 2 hours. Eggs were candled after 9 days of incubation to identify embryonic development. Non-fertilized eggs were removed (see Table 2 for number of eggs and fertilization). From day 15 onwards, the incubation temperature was set at 37.5°C, the humidity at 75%, and the eggs were no longer turned. After 15 days of incubation, eggs were placed in separate compartments (5.5 x 5.5 x 5 cm) on hatching trays. The individual compartments allowed us to identify which chick hatched from which egg. The compartment walls were made of transparent Plexiglas and the bottom of each hatching tray was made of mesh wire, allowing air flow and olfactory and acoustic communication between the chicks.

All eggs hatched after 17 ± 1 days of incubation. Hatchlings were removed from the incubator once their feathers had dried (ca. 2 hours after hatching) and weighed to the nearest 0.1 g. A blood sample (max. 50 μ l or about 0.5% of body weight; <0.8% does not appear to have long-term effects on adult or developing birds; Sheldon et al. 2008) was taken for assignment of parentage. Blood sampling was done by piercing the jugular vein with a sterile 27-gauge needle and collecting the blood in heparinized capillaries (BRAND GMBH + CO KG, Wertheim, Germany).

Parentage assignment

F2 hatchling blood was centrifuged for 10 minutes at 2000 x g. Blood cells were diluted 1:2 with phosphate buffer saline (10 mM PBS+6 mM EDTA, pH 7.4) and stored at -20°C. We used a small sample of blood from the stress protocol or GnRH challenge from the adult F1 females. Genomic DNA was obtained by a phenol/chloroform or Chelex extraction (Walsh et al., 1991). Parentage was manually assigned after genotyping all parents and offspring at 22 microsatellite loci using fluorescently labelled primers, as described previously (Langen et al., 2017).

Stress protocol and GnRH challenge

The stress protocol and the GnRH challenge were performed after collecting the F2 generation eggs to exclude effects on reproduction. The stress protocol took place on days 90 - 91. All birds were tested between 09:20 am - 12:30 pm and corticosterone levels did not change significantly during that period ($\chi^2_{(1)} = 0.30$, $p = 0.58$). After catching the birds from their home cages, a blood sample was taken within 3 minutes to determine baseline plasma corticosterone concentrations by puncturing the ulnar vein with a sterile needle and collecting 200 - 300 μ l blood in heparinised capillaries (BRAND GMBH + CO KG, Wertheim, Germany). After taking the baseline sample, the birds were restrained for 10 minutes by placing them in a cotton bag (Ecotone, 25 x 30 cm). A second blood sample was taken after the 10-minute restraint period to determine the female's corticosterone response (in total, 2 x 200 - 300 μ l blood was collected on the days of the stress protocol and the GnRH challenge, or about 0.18% - 0.28% of body weight at those ages; <0.8% does not appear to have long-term effects on adult or developing birds; Sheldon et al., 2008).

The GnRH challenge took place on days 96 - 97 while all females were laying eggs and thus assumed to be responsive to GnRH (Jawor et al., 2006; Peluc et al., 2012). All birds were tested between 09:25 am - 12:30 pm. As in the stress protocol, birds were caught, and a blood sample was taken from the ulnar vein within 3 minutes to determine baseline plasma androgen concentrations. After the baseline sample was taken, the females were injected in the pectoral muscle with 5 μ g (based on Peluc et al., 2012) chicken GnRH-I (H-3106, APC number 54-8-23, CAS No: 47922-48-5, Bachem, Bubendorf, Switzerland, formerly also sold as Sigma-L0637) dissolved in 50 μ l PBS, and returned to their home cages. Thirty minutes post injection, the birds were caught again, and a second blood sample was taken to determine the female's plasma androgen concentration in response to GnRH.

Hormone analysis

Blood samples from the stress protocol and the GnRH challenge were kept on ice for a maximum of two hours after sampling and then centrifuged for 10 minutes at 2000 x g. Following centrifugation, plasma was collected and frozen at -20°C.

Plasma corticosterone concentrations were determined using a commercial corticosterone radioimmunoassay kit (MP Biomedicals, Orangeburg, USA, cat. no. 07-102102). Cross-reactivity of the kit antibody was 0.34% for desoxycorticosterone, 0.1% for testosterone, and less than 0.1% for all other steroids tested (as reported by the manufacturer). Samples were measured together with quail plasma samples from other experiments and were distributed over 10 assays with an average intra-assay coefficient of variation (CV) of 4.78%, and an inter-assay CV of 7.13% (based on a chicken plasma pool and 2 kit controls measured in duplicate in each assay). Across assays, samples were balanced for treatment.

Plasma androgen concentrations were determined using a commercial testosterone enzyme immunoassay kit (Demeditec Diagnostics GmbH, Kiel, Germany, cat. no. DES6622). Cross-reactivity of the kit antibody was 23.3% for 5 α -dihydrotestosterone, 1.6% for androstenedione, and less than 0.1% for other tested steroids (as reported by the manufacturer). Samples were measured together with quail plasma samples from other experiments and were distributed over 9 assays with an average intra-assay coefficient of variation (CV) of 4.38% (based on all plasma samples measured in duplicate), and an inter-assay CV of 13.82% (based on 2 control plasma pools measured in each of the 9 assays). Across assays, samples were balanced for treatment.

Statistical analysis

Data were analysed using R 3.4.3 (R Core Team, 2017), package lme4 (Bates et al., 2015). General linear mixed models were fitted for body mass, mass around egg collection, egg mass, F2 mass at hatching and plasma hormones. Analysis of egg laying rate (eggs/female/day between day 80 and day 87), fertilization and hatching success was done using generalised linear mixed models with a binomial error distribution and logit link function. To control for the non-independence of F1 offspring from the same P0 mother, we always included P0 mother as a random effect. We also included a random effect of F1 female nested within P0 mother for repeated measurements from the same F1 female (body mass, egg laying rate, fertilization and hatching success and plasma hormones).

All models included P0 social environment, F1 social environment and their interaction as fixed effects. Models analysing plasma hormones included an additional fixed effect of sample, and its two-way and three-way interaction with the P0 and F1 social environment. For the GnRH challenge, all females received the same amount of GnRH, without adjustment of the dosage for individual body mass. To investigate whether body mass affected circulating androgen levels or the response to the GnRH injection, we ran additional GnRH models including female mass as a covariate. Models analysing body mass included a linear, quadratic and cubic effect of age in days ($\text{day} + \text{day}^2 + \text{day}^3$) to model the non-linear relationship between age and mass. In addition, the two-way and three-way interactions between ($\text{day} + \text{day}^2 + \text{day}^3$) and the P0 and F1 social environment were included. The female's age in days was centered around the mean age within our dataset by subtracting 45 from each age. The intercept and main effects of the models therefore represent the estimated weight at day 45. We tested whether effects on F1 female mass could explain differences in F2 egg mass by including F1 females mass at day 90 (close to the period of egg collection) as a covariate in the model. Similarly, we included egg mass as a covariate in models testing effects on F2 mass at hatching. We also tested whether effects on mass at hatching depended upon offspring sex.

We started out with the full models, including all interactions, and then stepwise excluded all non-significant predictors or interactions ($p > 0.05$), except for the main parameters of interest, i.e. social treatment, age in days ($\text{day} + \text{day}^2 + \text{day}^3$; for body mass) and sample number (for hormonal responses: baseline and post-restraint or post-GnRH injection samples). Interactions were always excluded before the main effects involved in the interaction. We determined the significance of fixed effects using likelihood ratio tests, comparing the models with and without the parameter of interest. Distributions of model residuals were visually assessed for normality and homoscedasticity using histograms and Q-Q plots. Plasma corticosterone concentrations were log10-transformed to achieve normality. The results of all models are reported in Table S2-S5, and the dataset used for analyses is reported in Table S1.

Results

Body mass, egg mass and offspring mass

Females housed in groups increased weight faster than females housed in pairs (own social environment*($\text{day} + \text{day}^2 + \text{day}^3$): $\chi^2_{(3)} = 21.94$, $p < 0.001$; Fig. 2A). In addition, there was a significant effect of the interaction between the P0 maternal social environment and F1 own social environment on female mass ($\chi^2_{(1)} = 4.14$, $p = 0.04$). The P0 social environment on its own or in interaction with age did not affect female weight ($\chi^2 < 0.46$, $p > 0.58$). The dataset was split according to maternal social environment and by day of weighing for further post-hoc testing. This analysis revealed that F1 group housing had a positive effect on body mass increase in daughters of pair-housed mothers and no effect on body mass increase in daughters of group-housed mothers (see Table S2 for more details). Furthermore, splitting the dataset by day revealed that the interaction effect between the maternal and own social environment on female mass was significant at day 37 and 44, with a nonsignificant trend at day 61. There was no significant interaction effect on days 24, 30, 90 and 97. From day 44 onwards, the F1 females own social environment significantly affected their body mass at each

time point, with group-housed females being heavier than pair-housed females. Detailed results of the post-hoc tests can be found in Table S2. Towards the end of the experiment, the separations of certain cages (see materials and methods) might have biased our results due to exclusion of heavier or lighter females. We therefore repeated the body mass analysis, including only data up to day 61 when most females were still included. In this analysis the effect of the interaction between the maternal and own social environment was borderline non-significant ($\chi^2_{(1)} = 3.78$, $p = 0.052$). The effect of the own social environment on weight increase was not significant (own social environment*(day + day² + day³): $\chi^2_{(3)} = 6.05$, $p = 0.11$; see Table S2 for more details).

At day 90, close to the period of egg collection for the F2 generation, females housed in groups were significantly heavier than females housed in pairs ($\chi^2_{(1)} = 6.44$, $p = 0.011$; Fig. 2B) and there was no longer an effect of the interaction with the P0 treatment ($\chi^2_{(1)} = 0.34$, $p = 0.56$). Additionally, females housed in groups laid heavier eggs than females housed in pairs ($\chi^2_{(1)} = 6.02$, $p = 0.014$; Fig. 2C) and the F2 offspring of females housed in groups were heavier at hatching than offspring of females housed in pairs ($\chi^2_{(1)} = 12.53$, $p < 0.001$, Fig. 2D). The P0 social environment did not affect egg mass, or F2 mass at hatching, and did not interact with the effects of the F1 social environment (all $\chi^2_{(1)}$ values < 1.36 , all p -values > 0.24 , all $\chi^2_{(3)}$ values < 4.51 , all p -values > 0.21 ; Fig. 2; Table S2-S3). We also found no sex differences in F2 offspring mass at hatching, and no effect of the interaction between F2 sex with the P0 maternal and the F1 own social environment (Table S3).

Egg mass was significantly positively correlated with F1 female mass at day 90 ($\chi^2_{(1)} = 5.59$, $p = 0.02$; Fig. 3A; Table S3). When controlling for female mass at day 90, the effect of the female's own social environment on egg mass was no longer significant ($\chi^2_{(1)} = 2.45$, $p = 0.12$; Table S3), suggesting that the effect of the F1 social environment on egg mass was mediated by effects on female body mass. Similarly, F2 mass at hatching was significantly positively correlated with egg mass ($\chi^2_{(1)} = 135.61$, $p < 0.001$; Fig. 3B; Table S3), and when controlling for egg mass, the effect of the female's own social environment on F2 mass at

hatching was no longer significant ($\chi^2_{(1)} = 1.39$, $p = 0.24$; Table S3). This suggests that the effect of the F1 social environment on F2 mass at hatching was mediated by the effects on egg mass.

Stress protocol and GnRH challenge

Females responded to the 10 minutes of restraint with a significant increase in plasma corticosterone concentrations ($\chi^2_{(1)} = 53.24$, $p < 0.001$; Fig. 4A), but the corticosterone response did not differ between females from different maternal or own social environments (maternal social environment * sample: $\chi^2_{(1)} = 1.69$, $p = 0.19$; own social environment * sample: $\chi^2_{(1)} = 1.69$, $p = 0.19$; Fig. 4A). There was also no effect of the interaction between the maternal and own social environment on the female's stress response (maternal social environment * own social environment * sample: $\chi^2_{(1)} = 2.33$, $p = 0.13$; Fig. 4A). Average plasma corticosterone concentrations were not affected by the female's own social environment, the maternal social environment, or their interaction (all $\chi^2_{(1)}$ values < 0.64 , all p values > 0.43 ; Fig. 4A; Table S4).

GnRH injections resulted in a significant increase in plasma androgen concentrations ($\chi^2_{(1)} = 26.43$, $p < 0.001$; Fig. 4B), but the androgen response to the GnRH challenge did not differ between females from different maternal or own social environments (maternal social environment * sample: $\chi^2_{(1)} = 0.22$, $p = 0.64$; own social environment * sample: $\chi^2_{(1)} = 0.96$, $p = 0.33$; Fig. 4B). The female's androgen response to GnRH was not affected by the interaction between the maternal and own social environment (maternal social environment * own social environment * sample: $\chi^2_{(1)} = 0.72$, $p = 0.40$; Fig. 4B). Average plasma androgen concentrations were not affected by the female's own social environment, the maternal social environment, or their interaction (all $\chi^2_{(1)}$ values < 0.55 , all p values > 0.46 ; Fig. 4B; Table S4). Female body mass at the time of the GnRH challenge significantly affected their response to the GnRH injection (sample * F1 body mass: $\chi^2_{(1)} = 7.80$, $p = 0.005$; Table S4). Post-hoc tests on the dataset split by sample revealed that there was a nonsignificant trend for female mass

to positively affect baseline androgen levels (F1 body mass: $\chi^2_{(1)} = 3.30$, $p = 0.07$; Table S4), but there was no effect of female body mass on response androgen levels (F1 body mass: $\chi^2_{(1)} = 0.89$, $p = 0.35$; Table S4). Including female body mass in the GnRH models did not change the effects of the maternal or own social environment. We therefore excluded female body mass from the final models to avoid potential confounding effects caused by multicollinearity (since female body mass was affected by the social environment, another predictor in the model).

Reproduction

Egg laying rates (eggs/female/day) were not affected by the maternal social environment ($\chi^2_{(1)} = 0.89$, $p = 0.35$; Fig. 5A), the F1 female's own social environment ($\chi^2_{(1)} = 0.11$, $p = 0.75$; Fig. 5A), or the interaction between the maternal and own social environment ($\chi^2_{(1)} = 0.01$, $p = 0.92$; Fig. 5A). Offspring from pair-housed mothers laid slightly more fertilized eggs than offspring from group-housed mothers, but the difference did not reach statistical significance ($\chi^2_{(1)} = 2.89$, $p = 0.09$; Fig. 5B). There was no effect of the F1 female's own social environment ($\chi^2_{(1)} = 1.08$, $p = 0.30$; Fig. 5B) or of the interaction between the maternal and own social environment on fertilization success ($\chi^2_{(1)} = 0.77$, $p = 0.38$; Fig. 5B).

The hatching success of fertilized eggs was higher for females housed in groups compared to females housed in pairs ($\chi^2_{(1)} = 4.07$, $p = 0.04$; Fig. 5C). The maternal social environment and its interaction with the female's own social environment did not affect hatching success of fertilized eggs ($\chi^2_{(1)} = 2.63$, $p = 0.11$ and $\chi^2_{(1)} = 0.13$, $p = 0.72$, respectively; Fig. 5C). Overall hatching rates (the proportion of all eggs collected for the F2 generation that hatched, i.e. including non-fertilized eggs) were not affected by the female's own social environment, the maternal social environment, or their interaction (all $\chi^2_{(1)}$ values < 1.88 , all p values > 0.17 ; Table S5).

Discussion

This study is the first to our knowledge to test for evidence of adaptive maternal effects and the underlying mechanisms in relation to social group size in a match-mismatch experiment across two generations in Japanese quail. Body mass of the F1 females was affected by their own social environment, as females housed in groups increased weight faster and ended up heavier compared to pair-housed females. Notably, however, body mass of the F1 females also depended on the interaction between the maternal and own social environment, which was due to an additional positive effect on mass in daughters of P0 pair-housed females only when they were housed in F1 groups. This interaction effect on mass disappeared by the time eggs for the F2 were collected (day 90). This suggests that offspring of pair-housed females housed in groups increased weight at an earlier age than offspring of group-housed females who caught up later. There was no effect of the P0 social environment on F1 mass before the F1 social treatment started (see also Langen et al., 2018). The positive effect on offspring body mass in the mismatched environment, at least for offspring of pair-housed females, contradicts the expectation of an adaptive maternal effect, since it does not suggest that offspring perform better in the environment matching the maternal one. A non-adaptive explanation may be a silver spoon effect due to increased maternal investment of pair-housed mothers resulting in a stronger positive effect of the group environment on their mass compared to offspring of group-housed mothers (Marshall and Uller, 2007; Uller et al., 2013). There was no evidence of a difference in P0 maternal investment since egg mass and yolk androgen levels did not differ. However, females housed in pairs had higher circulating androgen levels (Langen et al. 2017) which may be associated with differences in other aspects of egg quality. To explain why a maternal effect may be context dependent, it has been suggested that more competitive or otherwise challenging conditions may be required to detect maternal effects on offspring phenotype (Benowitz-Fredericks et al., 2015; Verboven et al., 2003). Offspring of pair-housed females may thus respond more strongly than offspring of group-housed females to the stimulating effect of the social group environment. While overall our results thus do not suggest

an adaptive effect, they emphasize the importance of investigating maternal effects under different environmental conditions in the offspring.

The interaction effect of the P0 and the F1 social environment on female mass disappeared by the time eggs for the F2 were collected, and at that point only the positive effect of the current group size on female mass remained. This effect can explain the larger egg size and hatching success and a positive maternal effect on F2 hatchling mass for group-housed females. The positive effects of group-housing on egg mass and offspring mass at hatching can ultimately have important fitness consequences because both are important predictors of offspring growth and survival (Krist, 2011; Williams, 1994). Our results thus strongly suggest that there is additional scope for adaptive maternal effects in relation to group size in Japanese quail and that the observed effects of the social environment on body mass have important consequences for egg and offspring quality.

The effects of pair-housing versus group-housing on females and their offspring differed between the P0 and F1 generations. In the P0 generation (Langen et al. 2017), female plasma androgen and corticosterone concentrations were affected but there were no effects on body mass, reproduction or F1 offspring mass at hatching. In contrast, the social environment of the F1 females affected body mass, reproduction, and F2 offspring mass, but not circulating androgen and corticosterone concentrations or the hormonal response to challenges. A possible explanation for these differences could be that the sex ratios within pairs and groups differed between the generations. Whereas males were continuously present in the female's social environment in the P0 generation, they were housed separately from the females in the F1 generation, and male-female interaction was only possible during the mating sessions. Pair-housing in the P0 generation likely resulted in more social stimulation by the male, leading to elevated female plasma androgen levels and a trend of higher plasma corticosterone (Langen et al., 2017). This effect by the male might have been diluted in the P0 group environment. In the F1 generation, female exposure to the male was standardized, explaining the absence of

a treatment difference in endocrine parameters and a stronger effect of group size on female mass. The contrasting effects of the P0 and the F1 social treatments may not only have been caused by the differences in sex ratio, but also by slight differences in timing between the P0 and F1 generation in the onset of the social treatments (day 29 in the P0 generation vs. day 24 in the F1 generation), the age at which females were first mated, the timing of sampling (for details see Fig. 1) and the number of females present.

F1 females that were housed in groups increased more in weight than pair-housed females and were heavier around the time of egg collection. This was unexpected since a negative correlation between group size or social density and growth or body mass has been reported in many animal species, including Japanese quail, likely due to increased competition for resources (Asghar Saki et al., 2012; Keeling et al., 2003; Onbaşlar and Aksoy, 2005). However, increased social stimulation can also lead to increased body mass, as demonstrated in European starlings (*Sturnus vulgaris*; Witter and Goldsmith, 1997), potentially because higher levels of social stimulation can increase food intake rates (Beauchamp, 1998; Hoppitt and Laland, 2008; Tolman, 1964). Since we did not measure female body composition, we do not know whether differences in weight between the social treatments were due to an overall increase in body mass or due to increased mass of specific tissues, such as the reproductive organs, which might be an explanation for the larger F2 egg mass, offspring mass and hatching success. Increased body mass is generally expected to be beneficial under higher social densities because it may increase female competitive abilities (Clutton-Brock and Huchard, 2013; Stockley and Bro-Jørgensen, 2011), and our results indicate that it can lead to increased reproductive investment, in line with previous findings (Christians, 2002; Drent and Daan, 1980; Lim et al., 2014; Ronget et al., 2018; Sockman et al., 2006). Other proximate explanations may be changes in feed conversion or metabolic rate, potentially in combination with maternal effects. Both (maternal) corticosterone and testosterone may affect metabolism and body mass (Dantzer et al., 2013; Groothuis et al., 2005; Sapolsky et al. 2000). However, even though the P0 maternal circulating hormones were affected, we did not find differences

in yolk hormone deposition (Langen et al 2017) or plasma steroids of the F1 females. Moreover, F1 group and pair females did not show different hormonal responses to the challenges, so that our measurements do not suggest that differences in weights were linked to hormonal differences. Since we were unable to determine social status, it is also unclear whether differences in social hierarchy within pairs and groups may have contributed to the effects on weight and other parameters. Finally, some cages had to be removed from the experiment due to aggression, and we cannot exclude the possibility that this may have contributed to the effect on weight, because the growth trajectories of the removed females may have differed. When analysing only weight data until day 61, when most cages were still included, the model estimated a similar effect of F1 group housing on weight as in the full dataset, even though it was less clear and no longer significant.

Egg laying rates were not affected by the maternal or the own social environment and fertilization success was not affected by the own social environment, but daughters from pair-housed mothers had a non-significantly higher proportion of fertilized eggs than daughters from group-housed mothers. This effect was small and did not reach statistical significance, but a similar trend to higher fertility of pair-housed mothers was seen in the P0 generation (Langen et al., 2017). This suggests a genetic or non-genetic maternal effect on fertility which should be further investigated as it is a core fitness component.

Effects on female mass and reproduction in the F1 generation did not correspond with changes in female endocrine parameters, suggesting that effects of the social environment on female mass and reproduction were not mediated by differences in female plasma androgens and corticosterone in our experiments. Vice versa, in the P0, hormone differences did not lead to reproductive differences. Other studies report non-significant, positive, and negative correlations between circulating androgens or glucocorticoids and measures of reproduction (e.g. egg production, Gerlach and Ketterson, 2013; Veiga and Polo, 2008; hatching success, de Jong et al., 2016; Schmidt et al. 2009; number of fledglings, Burtka et al., 2016; O'Neal et al. 2008; Ouyang et al., 2011), suggesting that the relationships are non-linear and can change

across contexts and over time (Bonier et al., 2009a; Hau and Goymann, 2015; Ouyang et al., 2011, 2013). Moreover, it is important to note that due to the exclusion of some groups as a result of aggression the sample size of group-housed females for the endocrine measurements became rather small at the end of the study when hormone measurements were taken (ranging from four to seven females).

Conclusions

We have shown that offspring development is affected by the maternal social environment, the offspring's own social environment and the interaction of both. The effects differ according to the trait of interest and time point of measurement. While F1 group housing generally had a positive effect on body mass, there was an additional positive effect on F1 body mass seen only when offspring of pair-housed females were housed in groups, suggesting that differences in P0 maternal investment modulated offspring response to its own environment. This result emphasizes the importance of considering the context under which maternal effects are studied and lends some support to the idea that maternal effects may be revealed better under more challenging or stimulating conditions. The interaction effect between the maternal and offspring social environment disappeared over time to be replaced by the effects of the F1 own social environment, which resulted in a maternal effect on the F2 generation that was independent of the P0 social environment. The observed changes in mass in the F1 and F2 generations are likely to have important consequences for performance and fitness, but their adaptive significance remains unclear. Effects of social group size on female physiology and reproduction differed between the P0 and the F1 generation most likely because the adult sex ratio did not remain constant over the generations. This might have led to differences in social stimulation between pairs and groups of both generations, potentially explaining why the effects of the matched and mismatched social conditions did not confirm expectations. Future studies of the adaptive maternal effects of the social environment and the underlying proximate mechanisms should assess the fitness consequences for offspring in more depth.

Furthermore, the importance of the type of social stimuli experienced (e.g. group size, adult sex ratio, intrasexual and intersexual interactions) should be investigated in more detail.

Acknowledgements

We thank Aline Bertin and her colleagues at INRA in Nouzilly, France for providing us with our first generation of Japanese quail and for their advice. We also thank Judith Hendriks, Elke Hippauf and Susanne Kirchhoff for their help in the lab, Irene de la Casa and Sarah Golücke for helping with the experimental procedures, the animal caretakers for looking after the birds, and Suzanne von Engelhardt for English editing of the manuscript. We gratefully acknowledge two anonymous reviewers for providing constructive comments on a previous version of the manuscript. Please note that the results in this paper are reproduced from the PhD thesis of Esther M.A. Langen (Bielefeld University, 2018).

Competing interests

The authors declare no competing interests.

Funding

This research was funded by grant support from the Volkswagen Foundation (Az 86 005, acquired by VCGJ). The funding source had no role in the study design, data collection or analysis, preparation of the manuscript, or the decision to submit the manuscript for publication.

Data availability

All data are within the paper and its supplementary data files.

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Figures

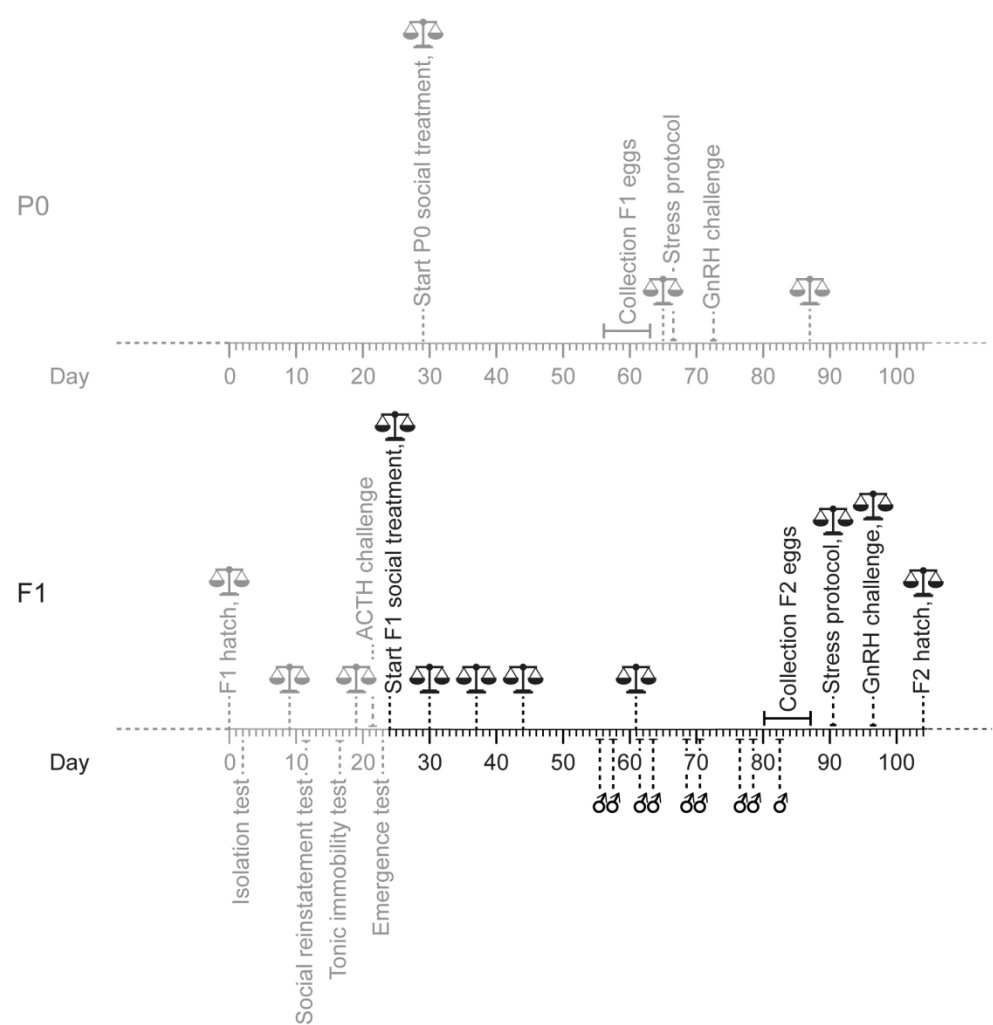


Fig. 1. Timeline of experimental procedures. Measurements in grey are not presented here, but some of these are published elsewhere (see Langen et al., 2017, 2018 for more information). Scale symbols indicate when animals were weighed. ♂ indicates when females and males were brought together for mating.

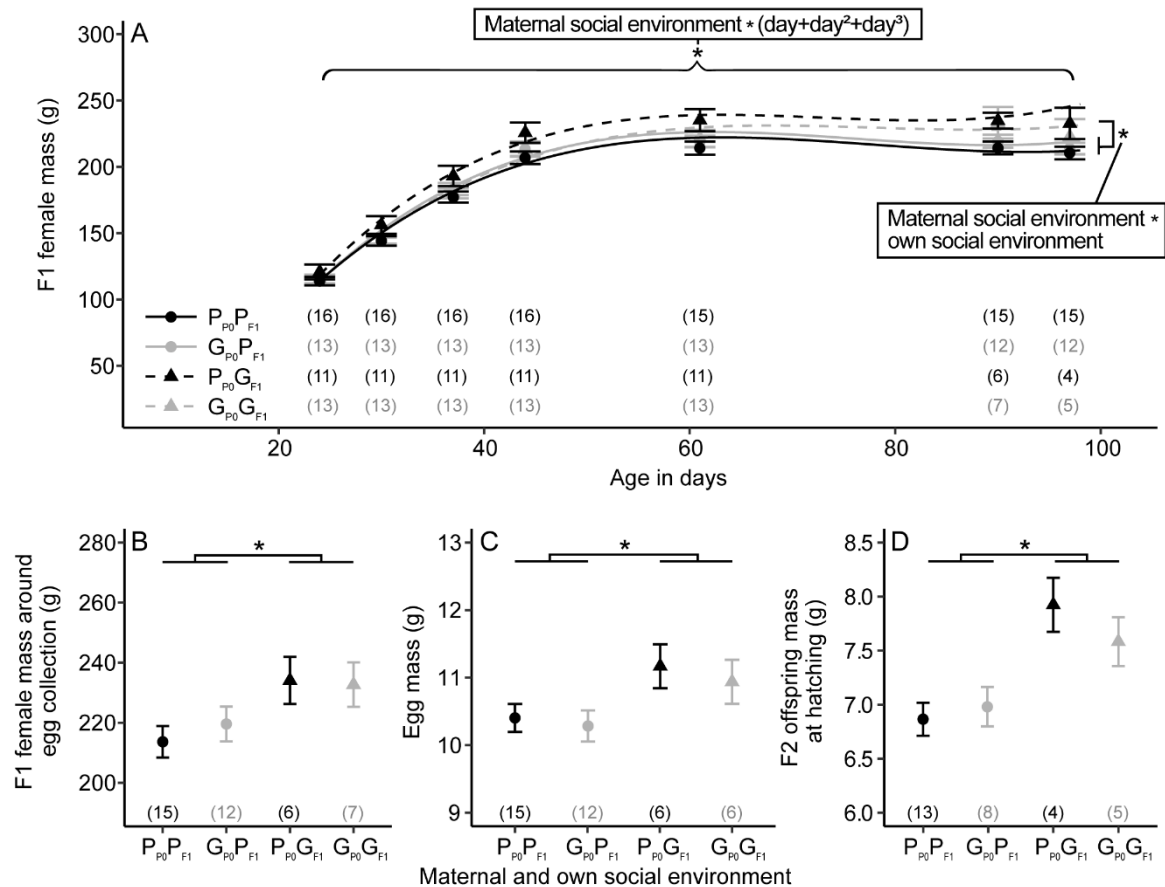


Fig. 2. Female body mass, mass around egg collection, egg mass and F2 offspring mass at hatching. A: Female body mass. Females housed in groups in the F1 (triangles and dashed lines) increased body mass faster than F1 females housed in pairs (circles and solid lines). In addition, F1 group housing had a positive effect on weight, but only in daughters of pair-housed females, not of group-housed females. B: average female mass around egg collection (day 90). Females housed in groups were significantly heavier than females housed in pairs. There was no effect of the maternal social environment or its interaction with the female's own social environment. C: egg mass. Females housed in groups laid significantly heavier eggs than females housed in pairs. There was no effect of the maternal social environment or its interaction with the female's own social environment. D: F2 offspring mass. Females housed in groups had significantly heavier F2 offspring than females housed in pairs. There was no effect of the maternal social environment or its interaction with the female's own social environment. Data shown in figure A are the raw means \pm 1 SEM, with lines indicating model predictions. Data shown in figure B, C and D are the estimated means \pm 1 SEM. Numbers between brackets indicate the number of F1 females included (for number of F2 offspring, see Table 2). * = $p < 0.05$ (see materials and methods section for details on which statistical methods were used).

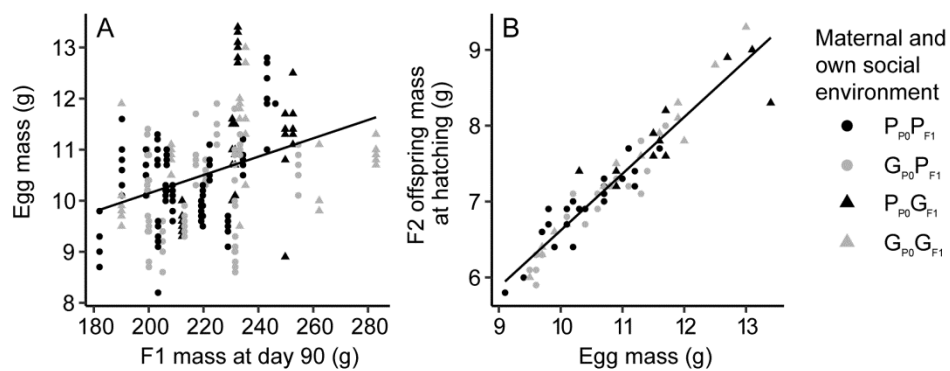


Fig. 3. A: relationship between F1 female mass around egg collection (day 90) and egg mass. B: relationship between egg mass and mass of the F2 offspring at hatching.

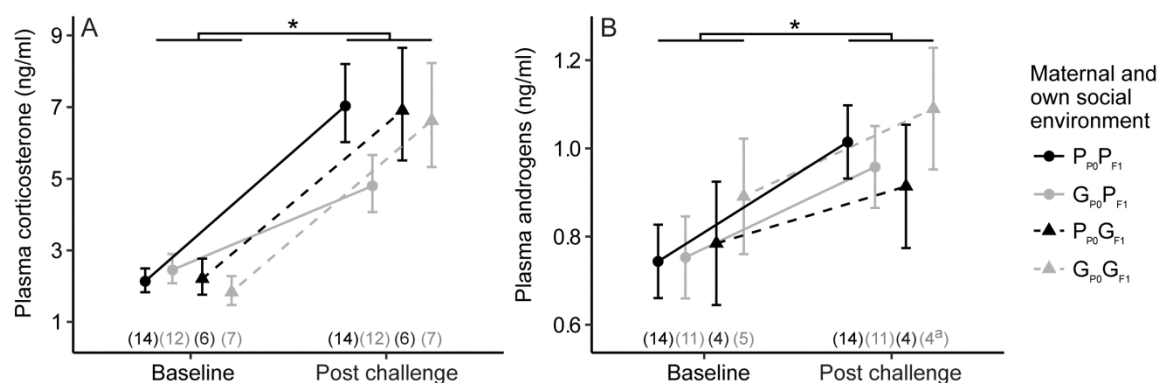


Fig. 4. Female plasma hormone levels. A: plasma corticosterone concentrations of F1 females 90-91 days old before and after being restrained for 10 minutes (back-transformed from log10). 10 minutes of restraint significantly increased plasma corticosterone concentrations, but there was no effect of the maternal or own social environment or their interaction on the increase, or on average plasma corticosterone concentrations. B: plasma androgen concentrations of F1 females 96-97 days old before and after an injection with 5 μ g GnRH. Androgen concentrations increased significantly in response to the GnRH injection, but there was no effect of the maternal or own social environment or their interaction on the increase, or on average plasma androgen concentrations. Data shown are the estimated means \pm 1 SEM. Numbers between brackets indicate the number of F1 females included. * = $p < 0.05$ (see materials and methods section for details on which statistical methods were used). ^a: insufficient plasma for one $G_{P_0}G_{F_1}$ female in the response sample.

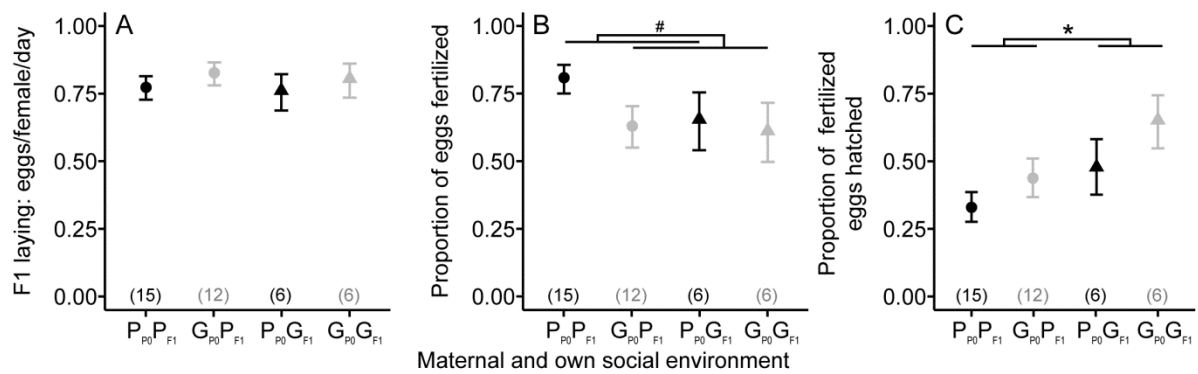


Fig. 5. Female reproduction. A: number of eggs laid per female per day. Egg laying rates were not affected by the maternal or own social environment or their interaction. B: proportion of eggs fertilized. There was a small nonsignificant effect of the maternal social environment, with offspring from pair-housed mothers laying slightly more fertilized eggs than offspring from group-housed mothers. Fertilization success was not affected by the own social environment or the interaction between the maternal and own social environment. C: hatching success of fertilized eggs. Hatching success was higher for females housed in groups compared to females housed in pairs. Hatching success was not affected by the maternal social environment or its interaction with the female's own social environment. Data shown are the estimated means \pm 1 SEM (back-transformed from logit). Numbers between brackets indicate the number of F1 females included (for number of eggs, see Table 2). # = 0.05 < p < 0.1; * = p < 0.05 (see materials and methods section for details on which statistical methods were used).

Tables

Table 1. Experimental groups and sample sizes (number of females in the two P0 social treatments and in the four combinations of F1 social treatments).

Maternal social environment	P0 females	Own social environment	F1 Females (P0 mothers)
P _{P0}	13	P _{F1} ^a	16 (11 ^c)
		G _{F1} ^b	11 (9 ^c)
G _{P0}	13	P _{F1} ^a	13 (10 ^d)
		G _{F1} ^b	13 (9 ^d)

^a: housed in 16 F1 pair cages; ^b: housed in 7 F1 group cages; ^c: 7 P0 pair-housed mothers contributed to both F1 pairs and F1 groups; ^d: 6 P0 group-housed mothers contributed to both F1 pairs and F1 groups.

Table 2. Sample sizes for F1 egg laying rates, egg mass, fertilization, hatching success and F2 offspring mass at hatching.

Maternal and own social environment	F1 females contributing to egg data	Eggs laid	Eggs fertilized	Eggs hatched	F1 females with F2 offspring hatching	F2 offspring
P _{P0} P _{F1}	15	93	73	24	13	24
G _{P0} P _{F1}	12	79 ^a	48	21	8	20 ^b
G _{P0} G _{F1}	6	38	23	15	5	15
P _{P0} G _{F1}	6	36	23	11	4	11

^a due to an oversight only 77 eggs were weighed. ^b 21 chicks hatched, but one chick was excluded from the mass measurements because of birth defects.

Table S1

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Table S2. General linear mixed model of effects of the maternal and F1 own social environment on female body mass.

Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	205.200	4.620	35.870	44.403			
Day * maternal social environment * own social environment ^a	0.002	0.254	286.900	0.006			
Day ² * maternal social environment * own social environment ^a	0.022	0.013	286.900	1.667	4.51	3	0.21
Day ³ * maternal social environment * own social environment ^a	-4.62*10 ⁻⁴	3.13*10 ⁻⁴	287.100	-1.474			
Day * maternal social environment ^a	0.030	0.126	286.900	0.236			
Day ² * maternal social environment ^a	0.003	0.007	286.900	0.459	0.46	3	0.93
Day ³ * maternal social environment ^a	-5.64*10 ⁻⁵	-1.54*10 ⁻⁴	286.900	-0.366			
Day * own social environment	0.168	0.128	287.000	1.313			
Day ² * own social environment	-0.002	0.007	286.900	-0.225	21.94	3	< 0.001
Day ³ * own social environment	7.25*10 ⁻⁵	1.57*10 ⁻⁴	287.100	0.460			
Maternal social environment * own social environment	-13.260	6.391	34.090	-2.076	4.14	1	0.04
Day	2.215	0.082	286.900	26.884			< 0.001
Day ²	-0.086	0.004	286.700	-19.739			< 0.001
Day ³	-8.97*10 ⁻⁴	9.93*10 ⁻⁵	286.800	9.038			< 0.001
Maternal social environment	3.648	6.506	34.100	0.561			0.58
Own social environment	14.370	4.723	42.220	3.043			0.004
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID ^b	85.45	9.244					
Maternal ID	159.47	12.628					
Residual	120.24	10.966					
Post-hoc tests; split by maternal social environment:							
Offspring of pair-housed mothers							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	205.800	5.636	15.520	36.513			
Day * own social environment	0.165	0.169	146.600	0.975			
Day ² * own social environment	-0.013	0.009	146.400	-1.466	20.77	3	< 0.001
Day ³ * own social environment	3.08*10 ⁻⁴	2.08*10 ⁻⁴	146.700	1.482			

Day	2.194	0.103	146.600	21.265				<0.001
Day ²	-0.083	0.005	146.300	-15.353				<0.001
Day ³	8.42*10 ⁻⁴	1.24*10 ⁻⁴	146.400	6.814				<0.001
Own social environment	15.530	3.685	31.050	4.214				<0.001
Random effects								
	Variance	Std.Dev.						
Maternal ID : F1 ID ^b	28.370	5.327						
Maternal ID	342.450	18.506						
Residual	102.540	10.126						
Offspring of group-housed mothers								
Fixed effects								
	Estimate	SE	df	t	χ ²	df	p	
(Intercept)	208.800	4.209	32.390	49.601				
Day * own social environment	0.164	0.191	140.400	0.858				
Day ² * own social environment	0.009	0.010	140.400	0.925	6.55	3	0.09	
Day ³ * own social environment	1.48*10 ⁻⁴	2.35*10 ⁻⁴	140.400	-0.632				
Day	2.241	0.129	140.300	17.373				<0.001
Day ²	-0.090	0.007	140.300	-13.013				<0.001
Day ³	9.66*10 ⁻⁴	1.57*10 ⁻⁴	140.300	6.172				<0.001
Own social environment	-1.544	5.494	34.330	-0.281				0.78
Random effects								
	Variance	Std.Dev.						
Maternal ID : F1 ID ^b	122.610	11.073						
Maternal ID	37.150	6.095						
Residual	134.390	11.593						
Post-hoc tests; split by own social environment								
Pair-housed F1 females								
Fixed effects								
	Estimate	SE	df	t	χ ²	df	p	
(Intercept)	202.400	4.154	19.270	48.727				
Day	2.215	0.077	168.700	28.641				
Day ²	-0.086	0.004	168.700	-21.038	476.62	3	< 0.001	
Day ³	8.98*10 ⁻⁴	9.31*10 ⁻⁵	168.700	9.640				
Maternal social environment	5.322	5.883	17.940	0.905	0.81	1	0.37	
Random effects								
	Variance	Std.Dev.						
Maternal ID : F1 ID ^b	85.190	9.230						
Maternal ID	101.430	10.070						
Residual	105.850	10.290						
Group-housed F1 females								
Fixed effects								
	Estimate	SE	df	t	χ ²	df	p	
(Intercept)	218.000	6.102	19.490	35.731				

	Day	2.383	0.106	117.600	22.496			
	Day ²	-0.088	0.006	117.600	-15.855	331.68	3	< 0.001
	Day ³	9.68*10 ⁻⁴	1.32*10 ⁻⁴	117.700	7.315			
	Maternal social environment	-11.430	8.379	17.280	-1.364	1.77	1	0.18
Random effects								
		Variance	Std.Dev.					
	Maternal ID : F1 ID ^b	63.110	7.944					
	Maternal ID	244.420	15.634					
	Residual	141.150	11.881					
Post-hoc tests; split by day								
Day 24								
Fixed effects								
		Estimate	SE	df	t	χ ²	df	p
	(Intercept)	116.295	3.561	28.387	32.662			
	Maternal social environment	-2.113	4.779	23.580	-0.442	0.2	1	0.66
	Own social environment	3.441	2.893	34.772	1.189	1.39	1	0.24
	Maternal social environment * own social environment ^a	-2.135	5.811	34.585	-0.367	0.13	1	0.72
Random effects								
		Variance	Std.Dev.					
	Maternal ID	95.840	9.790					
	Residual	88.530	9.409					
Day 30								
Fixed effects								
		Estimate	SE	df	t	χ ²	df	p
	(Intercept)	149.121	4.292	27.343	34.741			
	Maternal social environment	-5.379	5.861	23.980	-0.918	0.83	1	0.36
	Own social environment	4.349	2.894	31.810	1.503	2.19	1	0.14
	Maternal social environment * own social environment ^a	-8.599	5.729	31.905	-1.501	2.19	1	0.14
Random effects								
		Variance	Std.Dev.					
	Maternal ID	171.760	13.106					
	Residual	83.810	9.155					
Day 37								
Fixed effects								
		Estimate	SE	df	t	χ ²	df	p
	(Intercept)	180.357	4.879	33.334	36.968			
	Maternal social environment	3.955	7.085	35.612	0.558			0.58
	Own social environment	13.919	5.018	33.335	2.774			0.009
	Maternal social environment * own social environment	-16.558	7.219	34.714	-2.294	5.00	1	0.03
Random effects								

	Variance	Std.Dev.					
Maternal ID	177.000	13.300					
Residual	135.300	11.630					
Day 44							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	208.473	5.542	32.531	37.617			
Maternal social environment	6.778	8.053	34.895	0.842			0.41
Own social environment	19.030	5.775	32.545	3.295			0.002
Maternal social environment * own social environment	-16.671	8.306	33.983	-2.007	3.88	1	0.049
Random effects							
	Variance	Std.Dev.					
Maternal ID	223.700	14.960					
Residual	179.900	13.410					
Day 61							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	220.301	5.640	28.335	39.064			
Maternal social environment	-2.699	7.355	21.428	-0.367	0.13	1	0.71
Own social environment	10.442	5.143	36.334	2.030	3.97	1	0.046
Maternal social environment * own social environment ^a	-18.76	9.835	36.251	-1.907	3.49	1	0.06
Random effects							
	Variance	Std.Dev.					
Maternal ID	187.900	13.710					
Residual	280.700	16.750					
Day 90							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	214.755	4.930	24.713	43.559			
Maternal social environment	3.520	6.680	19.953	0.527	0.28	1	0.60
Own social environment	16.726	6.318	32.297	2.648	6.44	1	0.011
Maternal social environment * own social environment ^a	-7.336	12.550	32.250	-0.585	0.34	1	0.56
Random effects							
	Variance	Std.Dev.					
Maternal ID	56.580	7.522					
Residual	321.820	17.939					
Day 97							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	210.825	4.915	21.503	42.898			
Maternal social	1.260	6.873	18.242	0.183	0.03	1	0.86

environment							
Own social environment	14.973	6.970	29.798	2.148	4.31	1	0.04
Maternal social environment * own social environment ^a	-12.447	13.816	29.726	-0.901	0.8	1	0.37
Random effects							
	Variance	Std.Dev.					
Maternal ID	63.770	7.985					
Residual	290.630	17.048					
Including only data until day 61 (when most cages were still together):							
Fixed effects							
	Estimate	SE	df	t	χ ²	df	p
(Intercept)	215.200	4.612	35.970	46.656			
Day * maternal social environment * own social environment ^a	0.137	0.635	211.100	0.215			
Day ² * maternal social environment * own social environment ^a	0.015	0.025	211.100	0.628	5.42	3	0.14
Day ³ * maternal social environment * own social environment ^a	-0.001	0.002	211.100	0.633			
Day * maternal social environment ^a	0.235	0.320	211.100	0.735			
Day ² * maternal social environment ^a	-0.010	0.012	211.100	-0.833	0.90	3	0.83
Day ³ * maternal social environment ^a	-9.47*10 ⁻⁴	0.001	211.100	-0.871			
Day * own social environment	0.349	0.321	211.100	1.086			
Day ² * own social environment	-0.008	-0.012	211.100	-0.603	6.05	3	0.11
Day ³ * own social environment	-5.50*10 ⁻⁴	0.001	211.100	-0.505			
Maternal social environment * own social environment	-11.920	6.019	33.010	-1.980	3.78	1	0.052
Day	3.374	0.162	211.100	20.812	<0.001		
Day ²	-0.139	0.006	211.100	-22.127	<0.001		
Day ³	-0.003	5.50*10 ⁻⁴	211.100	-6.064	<0.001		
Maternal social environment	2.455	6.464	33.780	0.380	0.71		
Own social environment	12.700	4.177	31.960	3.041	0.005		
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID ^b	75.400	8.683					
Maternal ID	168.620	12.985					
Residual	78.430	8.856					
Post-hoc tests; split by maternal social environment:							
Offspring of pair-housed mothers							
Fixed effects							

	Estimate	SE	df	t	χ^2	df	p
(Intercept)	215.100	5.642	16.790	38.127			
Day	3.245	0.233	107.100	13.931			
Day ²	-0.134	0.009	107.200	-14.773	361.07	3	<0.001
Day ³	-0.003	7.90*10 ⁻⁴	107.100	-3.602			
Own social environment	11.990	3.213	15.000	3.733	10.31	1	0.001
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID ^b	33.720	5.807					
Maternal ID	326.380	18.066					
Residual	82.450	9.080					
Offspring of group-housed mothers							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	218.200	4.080	30.540	53.488			
Day	3.509	0.224	104.000	15.653			
Day ²	-0.145	0.009	104.000	-16.674	356.94	3	<0.001
Day ³	-0.004	7.61*10 ⁻⁴	104.000	-5.501			
Own social environment	-0.166	4.606	21.720	-0.036	0.001	1	0.97
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID ^b	105.100	10.252					
Maternal ID	49.710	7.050					
Residual	73.600	8.579					
Post-hoc tests; split by own social environment							
Pair-housed F1 females							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	211.100	4.114	20.640	51.296			
Day	3.215	0.197	115.000	16.290			
Day ²	-0.136	0.008	115.000	-17.732	398.72	3	<0.001
Day ³	-0.003	0.001	115.000	-4.617			
Maternal social environment	4.514	5.687	17.750	0.794	0.62	1	0.43
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID ^b	116.67	10.801					
Maternal ID	68.99	8.306					
Residual	63.58	7.973					
Group-housed F1 females							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	227.100	6.075	21.570	37.387			
Day	3.564	0.260	96.000	13.707			
Day ²	-0.143	0.010	96.000	-14.231	312.08	3	<0.001
Day ³	-0.004	0.001	96.000	-4.124			

Maternal social environment	-11.110	8.127	17.220	-1.367	1.77	1	0.18
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID^b	67.49	8.215					
Maternal ID	227.08	15.069					
Residual	91.41	9.561					

Estimates are given on the original scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. Factors included in the final model are presented in bold.

^a estimates and statistics are from the last model that still included the interaction.

^b F1 ID nested within maternal ID.

Table S3. General linear mixed model of effects of the maternal and F1 own social environment on egg mass and offspring mass at hatching.

Effects on egg mass:						
Fixed effects						
	Estimate	SE	χ^2	df	p	
(Intercept)	10.412	0.193				
Maternal social environment * own social environment ^a	-0.115	0.556	0.04	1	0.84	
Maternal social environment	-0.153	0.257	0.35	1	0.55	
Own social environment	0.711	0.278	6.02	1	0.01	
Random effects						
	Variance	Std.Dev.				
Maternal ID : F1 ID ^b	0.595	0.772				
Maternal ID	0.000	0.000				
Residual	0.258	0.508				
Including F1 female mass at day 90 as a covariate:						
Fixed effects						
	Estimate	SE	χ^2	df	p	
(Intercept)	6.902	1.341				
Maternal social environment * own social environment ^a	0.003	0.502	0.00	1	0.995	
Maternal social environment	-0.158	0.264	0.33	1	0.56	
Own social environment	0.439	0.275	2.45	1	0.12	
F1 female mass at day 90	0.016	0.006	5.59	1	0.02	
Random effects						
	Variance	Std.Dev.				
Maternal ID : F1 ID ^b	0.430	0.655				
Maternal ID	0.091	0.302				
Residual	0.258	0.508				
Effects on offspring mass at hatching:						
Fixed effects						
	Estimate	SE	χ^2	df	p	
(Intercept)	6.926	0.145				
Maternal social environment * own social	-0.261	0.543	0.23	1	0.63	

environment* F2 sex					
Maternal social environment * F2 sex	-0.138	0.262	0.27	1	0.60
Own social environment* F2 sex	-0.115	0.269	0.18	1	0.67
Maternal social environment * own social environment ^a	-0.457	0.413	1.19	1	0.28
Maternal social environment	-0.032	0.199	0.03	1	0.87
Own social environment	0.830	0.211	12.53	1	< 0.001
F2 sex	-0.116	0.131	0.78	1	0.38
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID^b	0.179	0.423			
Maternal ID	2.40*10⁻¹⁶	1.55*10⁻⁸			
Residual	0.228	0.477			
Including egg mass as a covariate:					
Fixed effects					
	Estimate	SE	χ ²	df	p
(Intercept)	-0.675	0.353			
Maternal social environment * own social environment ^a	0.137	0.117	1.36	1	0.24
Maternal social environment	-0.036	0.078	0.21	1	0.65
Own social environment	0.077	0.065	1.39	1	0.24
Egg mass	0.730	0.033	135.61	1	< 0.001
Random effects					
	Variance	Std.Dev.			
Maternal ID : F1 ID^b	0.000^c	0.000^c			
Maternal ID	0.013	0.112			
Residual	0.042	0.205			

Estimates are given on the original scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. Factors included in the final model are presented in bold.

^a estimates and statistics are from the last model that still included the interaction.

^b F1 ID nested within maternal ID.

^c variance parameters estimated as zero in the model.

Table S4. General linear mixed model of effects of the maternal and F1 own social environment on female plasma hormone levels in the restraint stress protocol and the GnRH challenge.

Female plasma corticosterone levels in the restraint stress protocol:							
Fixed effects							
	Estimate	SE	χ^2	df	p		
(Intercept)	0.364	0.055					
Time of day ^a	0.420	0.762	0.30	1	0.58		
Maternal social environment * own social environment * sample ^a	0.288	0.186	2.32	1	0.13		
Maternal social environment * own social environment ^a	0.008	0.118	0.005	1	0.95		
Own social environment * sample ^a	0.126	0.096	1.69	1	0.19		
Maternal social environment * sample ^a	-0.121	0.092	1.69	1	0.19		
Maternal social environment	-0.056	0.067	0.64	1	0.42		
Own social environment	0.012	0.059	0.04	1	0.85		
Sample	0.451	0.047	53.24	1	< 0.001		
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID^b	0.005	0.072					
Maternal ID	0.008	0.091					
Residual	0.043	0.208					
Female plasma androgen levels in the GnRH challenge:							
Fixed effects							
	Estimate	SE	χ^2	df	p		
(Intercept)	0.751	0.076					
Maternal social environment * own social environment * sample ^a	0.136	0.159	0.72	1	0.40		
Maternal social environment * sample ^a	-0.032	0.069	0.22	1	0.64		
Maternal social environment * own social environment ^a	0.161	0.194	0.55	1	0.46		
Own social environment * sample ^a	-0.079	0.080	0.96	1	0.33		
Maternal social environment	0.020	0.104	0.04	1	0.85		
Own social environment	0.044	0.100	0.17	1	0.68		
Sample	0.223	0.035	26.43	1	< 0.001		
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID^b	0.045	0.213					
Maternal ID	0.018	0.135					
Residual	0.020	0.143					
Including female mass as a covariate:							
Fixed effects							
	Estimate	SE	df	t	χ^2	df	p
(Intercept)	-0.744	0.559	41.512	-1.331			
Maternal social environment * own social environment * sample * F1 body mass ^a	-0.002	0.007	33.208	-0.271	0.07	1	0.79
Maternal social environment * own social environment * F1	-0.007	0.010	33.460	-0.743	0.54	1	0.46

body mass ^a							
Maternal social environment * sample * F1 body mass ^a	-0.003	0.003	33.205	-0.869	0.75	1	0.39
Maternal social environment * own social environment * sample ^a	0.087	0.145	33.714	0.602	0.36	1	0.55
Own social environment * sample * F1 body mass ^a	0.002	0.003	33.206	0.692	0.48	1	0.49
Own social environment * sample ^a	-0.012	0.076	33.677	-0.152	0.02	1	0.88
Maternal social environment * F1 body mass ^a	-0.002	0.005	33.058	-0.497	0.20	1	0.66
Maternal social environment * own social environment ^a	0.074	0.120	34.477	0.370	0.14	1	0.71
Maternal social environment * sample ^a	-0.038	0.062	33.429	-0.612	0.37	1	0.54
Own social environment * F1 body mass ^a	-0.007	0.005	33.987	-1.491	2.15	1	0.14
Sample * F1 body mass	-0.005	0.002	33.230	-2.964	7.80	1	0.005
Maternal social environment	0.030	0.089	34.150	0.332	0.11	1	0.74
Own social environment	-0.046	0.106	34.463	-0.492	0.17	1	0.68
F1 body mass	0.007	0.003	41.387	2.685			0.006
Sample	1.273	0.356	33.220	3.579			0.001
Random effects							
	Variance	Std.Dev.					
Maternal ID : F1 ID ^b	0.058	0.241					
Maternal ID	1.17*10 ⁻¹⁵	3.42*10 ⁻⁸					
Residual	0.016	0.127					
Post-hoc tests; split by sample:							
Baseline							
Fixed effects							
	Estimate	SE	df	t	χ ²	df	p
(Intercept)	-0.560	0.621	33.440	-0.901			
Maternal social environment	0.037	0.113	17.108	0.326	0.10	1	0.75
Own social environment	-0.005	0.122	29.814	-0.045	0.002	1	0.97
F1 body mass	0.006	0.003	33.770	2.104	3.30	1	0.07
Random effects							
	Variance	Std.Dev.					
Maternal ID	0.015	0.123					
Residual	0.078	0.280					
Post challenge							
Fixed effects							
	Estimate	SE	df	t	χ ²	df	p
(Intercept)	0.531	0.488	33.000	1.089			
Maternal social environment	0.011	0.083	33.000	0.134	0.02	1	0.89
Own social environment	-0.051	0.101	33.000	-0.504	0.25	1	0.62
F1 body mass	0.002	0.002	33.000	0.950	0.89	1	0.35
Random effects							
	Variance	Std.Dev.					

Maternal ID	0.000^c	0.000^c
Residual	0.065	0.237

Estimates for plasma corticosterone levels are given on the log10 scale, estimates for plasma androgen levels are given on the original scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. The baseline sample (before restraint or the GnRH injection) is coded as 1, the post-restraint/challenge sample is coded as 2. Factors included in the final models are presented in bold.

^a estimates and statistics are from the last model that still included the factor/interaction.

^b F1 ID nested within maternal ID.

^c variance parameters estimated as zero in the model.

Table S5. Generalized linear mixed model of effects of the maternal and F1 own social environment on egg laying rates, fertilization success, hatching success of fertilized eggs and overall hatching rates (the proportion of all eggs collected for the F2 generation that hatched, i.e. including non-fertilized eggs).

Effects on egg laying rates:						
Fixed effects:						
	Estimate	SE	χ^2	df	p	
(Intercept)	1.241	0.244				
Maternal social environment * own social environment ^a	-0.071	0.626	0.01	1	0.92	
Maternal social environment	0.309	0.327	0.89	1	0.34	
Own social environment	-0.105	0.327	0.10	1	0.75	
Random effects:						
	Variance	Std.Dev.				
Maternal ID : F1 ID ^b	0.008	0.090				
Maternal ID	0.111	0.334				
Effects on fertilization success:						
Fixed effects:						
	Estimate	SE	χ^2	df	p	
(Intercept)	1.318	0.313				
Maternal social environment * own social environment ^a	0.721	0.814	0.77	1	0.38	
Maternal social environment	-0.673	0.389	2.89	1	0.09	
Own social environment	-0.432	0.416	1.08	1	0.30	
Random effects:						
	Variance	Std.Dev.				
Maternal ID : F1 ID ^b	0.597	0.773				
Maternal ID	0.000 ^c	0.000 ^c				
Effects on the hatching success of fertilized eggs:						
Fixed effects:						
	Estimate	SE	χ^2	df	p	
(Intercept)	-0.745	0.236				
Maternal social environment * own social environment ^a	0.253	0.716	0.13	1	0.72	
Maternal social environment	0.536	0.324	2.63	1	0.11	
Own social environment	0.744	0.356	4.07	1	0.04	
Random effects:						
	Variance	Std.Dev.				
Maternal ID : F1 ID ^b	0.001	0.038				
Maternal ID	0.000 ^c	0.000 ^c				
Effects on overall hatching rates:						
Fixed effects:						
	Estimate	SE	χ^2	df	p	
(Intercept)	-1.193	0.275				
Maternal social environment * own social environment ^a	0.308	0.633	0.24	1	0.63	
Maternal social environment	0.188	0.334	0.32	1	0.57	
Own social environment	0.446	0.318	1.88	1	0.17	

Random effects:		
	Variance	Std.Dev.
Maternal ID : F1 ID^b	0.000^c	0.000^c
Maternal ID	0.122	0.350

Estimates are given on the logit scale. Maternal and F1 pair-housing is coded as 0, group-housing is coded as 1. Factors included in the final model are presented in bold.

^a estimates and statistics are from the last model that still included the interaction.

^b F1 ID nested within maternal ID.

^c variance parameters estimated as zero in the model.